Pharmacokinetics and Pharmacogenetics of Metronomic Chemotherapy

16

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Abstract

Despite the numerous preclinical and clinical studies that have been conducted on metronomic chemotherapy in the past 10 years, few pharmacokinetic and pharmacogenetics data on this dosing regimen are available. Indeed, only the pharmacokinetics of metronomically administered drugs, such as irinotecan, UFT, and vinorelbine, have been described in patients, but no data are available on the most widely explored agents in such an approach like cyclophosphamide or capecitabine. Methodological issues and the neglected importance of the relationship between plasma concentrations of metronomically administered chemotherapeutic drugs (and their active metabolites) contributed to the absence of data on the commonly used 50 mg/day cyclophosphamide schedule. Moreover, few data are available on the pharmacogenetics of metronomic chemotherapy, and, although some objective responses have been obtained in various tumors, it remains largely unknown which genetic backgrounds could affect or predict the clinical response of patients. Trials integrating pharmacokinetic and pharmacogenetics research are necessary to better evaluate the clinical benefit of metronomic chemotherapy.

16.1 Introduction

The behavior and characteristics of chemotherapeutic drugs are quite diverse. The study of pharmacokinetics (the dose–concentration relationship) and pharmacodynamics (the concentration–response relationship) of chemotherapeutic drugs reveals

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this diverse behavior and sheds light on the different patterns of drug action. The knowledge base in pharmacokinetics and pharmacodynamics has grown considerably over the last years, and it has enabled seemingly counterintuitive concentration–response relationships to be understood (e.g., the antiangiogenic activity of low-dose metronomic chemotherapeutic drugs). The combination of the pharmacokinetic characteristics with the pharmacodynamic properties of a chemotherapeutic drug response may provide an almost complete knowledge of the dose–response relationship and, above all, can allow one to estimate the possible drug response at any dose, including at metronomic doses.

Chemotherapeutic drug action begins with the administration of the compound and concludes with the pharmacological response, which can be a beneficial and/or an adverse reaction. The dose, the frequency of administration, and the route of administration can permit the optimization of the onset, intensity, duration, and quality of therapeutic effects for a particular tumor type and the minimization of any harmful effects of the drug [1]. Thus, the design of optimum dosing regimens requires a deep understanding of the processes and of the steps that translate the administration of the drug into the pharmacological response. It also requires an understanding of how the administration–response relationship may be influenced by patient characteristics, as well as other conditions that may appear during the chemotherapy regimen. These include the age and gender of the patient, genetic factors (i.e., single-nucleotide polymorphisms) [2], concurrent medications, and changes in the tumor population being treated over time (i.e., onset of resistance) [3].

Using pharmacokinetics and pharmacogenetics, it should be possible to identify patients who will respond better to therapy and those at risk of rapidly developing drug resistance or of suffering from significant toxicity. In this regard, it needs to be pointed out that the dosing of metronomic chemotherapy remains largely empirical in the absence of validated clinical surrogate markers and pharmacokinetic drug monitoring for the treatment effects. Such pharmacokinetic data and pharmacodynamic markers are emerging in early-phase, pilot clinical studies (e.g., AUCs and circulating endothelial progenitor cells) [4–6], but their value in randomized phase III clinical studies remains questionable.

16.2 Pharmacokinetics of Metronomic Chemotherapy Regimens

Despite the growing amount of preclinical studies and clinical trials that have been conducted in the past 10 years [7, 8], few pharmacokinetic data of these schedules are currently available. Indeed, at the moment, only the pharmacokinetics of metronomic irinotecan, topotecan, vinorelbine, UFT, paclitaxel, and temozolomide have been described in patients [4, 9–14], but no data about the most widely explored agents in such an approach like cyclophosphamide or capecitabine have been provided. This lack of information may limit the clinical use and the efficacy of the metronomic regimens. Moreover, the variability in any of the pharmacokinetic

parameters (e.g., the peak concentration) may affect the impact of the drug. This can typically be observed in patients with organ dysfunction where the inability to either metabolize or excrete the drug will lead to unexpected drug effects [1]. A good pharmacokinetic assessment of the drugs administered metronomically is therefore the first and most important step in designing an individual treatment regimen that will maximize the antitumor drug benefit.

16.2.1 Metronomic Camptothecins

16.2.1.1 Irinotecan

Despite abundant information about the pharmacology of irinotecan [15], and of its active metabolite SN-38, on cancer cells using different therapeutic approaches, no data were available on the clinical effects of metronomic irinotecan administration until 2008. The pharmacokinetics of metronomic irinotecan (and its active metabolite SN-38) was performed, for the first time, in twenty patients with metastatic colorectal carcinoma, heavily pretreated both with irinotecan- and oxaliplatin-based chemotherapies at different dose levels [9]. This pilot study was defined and based on a previous pharmacokinetic experience with infusional schedule of irinotecan published by Falcone and colleagues [16]. The three different dose levels of metronomic irinotecan (infused continuously without breaks) were chosen starting from a reduction of 75 % of the maximum tolerable dose of irinotecan when infused continuously over 21 days every 28 days, as reported by Herben et al. [17]. The sample size ranged from a minimum of five to a maximum of eight patients *per* group which was sufficient to find a statistical difference between SN-38 (the active metabolite of irinotecan) pharmacokinetic parameters of each dose level [9]. The main pharmacokinetic parameters of irinotecan and its metabolites are reported in Table 16.1, whereas the mean plasma profiles of irinotecan, SN-38, and SN-38-glucuronide (the inactive form of SN-38) at the different infusion schedules are shown in Fig. 16.1a–c, respectively. Pharmacokinetic analysis demonstrated that the concentration at the steady state (C_{ss}) of SN-38 ranged from 1.00 ± 0.52 to 3.33 ± 0.96 ng/ml and was compatible with the antiangiogenic concentrations found in preclinical studies [18]. As expected, the C_{ss} of SN-38-glucuronide were higher than the ones of SN-38. Moreover, pharmacokinetic analysis showed an increased metabolism of irinotecan into the active metabolite SN-38 when higher doses were administered (Fig. 16.1b), a clear indication that such a process at these dose levels was not saturated. Thus, the mean AUC value of SN-38 was significantly lower at the irinotecan 1.4 mg/m^2/day dose than at the 2.8 and 4.2 mg/m^2/day doses (Table 16.1), and significant differences were found between the C_{max} values of SN-38 and SN-38glu at different irinotecan doses [9].

16.2.1.2 Topotecan

Topotecan has excellent antiangiogenic properties when administered on a metronomic schedule in preclinical models [19–22]. Various dosing schedules of oral topotecan have been evaluated in phase I studies, establishing the maximum

Table 16.1 Pharmacokinetic parameters of irinotecan, SN-38, and SN-38-glucuronide at the doses of irinotecan 1.4, 2.8, and 4.2 mg/m^2/day in 20 colorectal cancer patients

	Mean ± SD		
	1.4 mg/m^2/day (n = 7)	2.8 mg/m^2/day (n = 5)	4.2 mg/m^2/day (n = 8)
Irinotecan			
AUC (day·ng/ml)	8,714.7 ± 1,564.3	13,877.7 ± 3,035.2	23,051.6 ± 5,002.3
CL (ml/day/m^2)	154.32 ± 28.4	170.31 ± 44.2	146.11 ± 25.3
$t_{1/2}\beta$ (h)	15.9 ± 5.1	20.2 ± 6.2	14.6 ± 3.2
C_{max} (ng/ml)	277.6 ± 125.3	382.9 ± 261.8	484.1 ± 243.1
C_{ss} (ng/ml)	143.1 ± 56.8	231.6 ± 101.4	390.0 ± 171.0[a,b]
T_{max} (day)	35	35	28
SN-38			
AUC (day·ng/ml)	59.43 ± 7.47	136.21 ± 10.61[c]	200.48 ± 12.26[a,b]
$t_{1/2}\beta$ (h)	18.9 ± 4.3	22.8 ± 6.7	19.9 ± 7.2
C_{max} (ng/ml)	1.62 ± 0.45	2.61 ± 1.07	4.03 ± 2.19[a]
C_{ss} (ng/ml)	1.00 ± 0.52	2.29 ± 0.87[c]	3.33 ± 0.96[a,b]
T_{max} (day)	42	35	35
SN-38-glucuronide			
AUC (day·ng/ml)	100.94 ± 8.82	268.86 ± 14.52[c]	430.10 ± 24.34[a,b]
$t_{1/2}\beta$ (h)	22.31 ± 5.1	17.4 ± 5.6	21.33 ± 6.8
C_{max} (ng/ml)	2.24 ± 0.58	5.59 ± 1.91[c]	8.45 ± 2.54[a, b]
C_{ss} (ng/ml)	1.63 ± 0.53	4.42 ± 1.98[c]	7.20 ± 1.59[a,b]
T_{max} (day)	49	42	42

AUC area under the time/concentration curve, $t_{1/2}\beta$ terminal half-life, C_{max} maximal plasma concentration, T_{max} time to peak, C_{ss} plasma concentration at the steady state

[a] $P < 0.05$ 4.2 vs. 1.4

[b] $P < 0.05$ 4.2 vs. 2.8

[c] $P < 0.05$ 2.8 vs. 1.4

tolerated dose to a 1.0 mg fixed daily dose for a metronomic regimen [23]. Indeed, Tillmanns and colleagues enrolled 16 heavily pretreated patients with various solid tumors in a phase I dose-ranging study consisted of 30-day treatment cycles of daily oral topotecan at dose levels of 0.25, 0.50, 0.75, 1.00, and 1.25 mg [23]. The dose-limiting toxicity was reached at 1.25 mg (i.e., two patients had grade 3 gastrointestinal toxicities), and the maximum tolerated dose was defined at 1.0 mg daily [23]. Interestingly, as previously noted for irinotecan, the topotecan C_{max} increased linearly with the dose and the median T_{max} was 2 h [23]. On the basis of these preliminary results, the same group implemented a combination of metronomic oral topotecan (0.25 mg daily) and oral pazopanib (400, 600, or 800 mg daily) in a phase I, dose-escalation study in female patients with gynecological tumors [12]. A preclinical pharmacokinetic study of metronomic topotecan plus pazopanib suggested the absence of drug–drug interactions [20]. The published clinical data supported these preclinical findings, although a large inter- and intrapatient variability was observed [12]. The median of topotecan *Cl*/*F*, *Vc*/*F*, and *k*a were 26.7 l/h, 144 l, and 1.04 h^{-1}, respectively. A mean topotecan C_{max} around 1 ng/ml was found at the 0.25 mg dose schedule, and the onset of the absorptive phase was delayed for several patients [12]. The authors indicated that a one-compartment model with

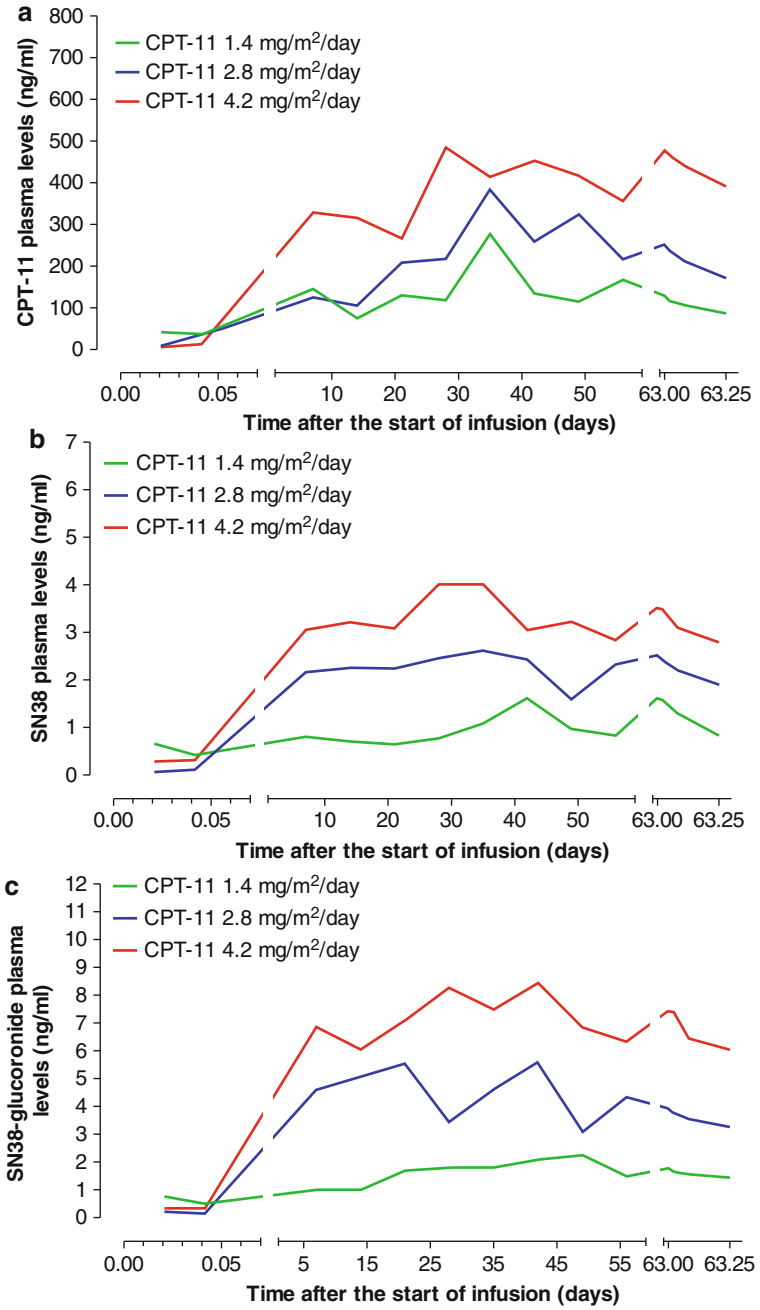

Fig. 16.1 Plasma levels of irinotecan (CPT-11) (**a**), SN-38 (**b**), and SN-38 glucuronide (**c**) in 20 mCRC patients receiving an i.v. continuous infusion of CPT-11 at three different dose levels. The continuous line represents the mean plasma concentrations

first-order absorption/lag-time and linear elimination from the central compartment adequately described the topotecan plasma concentrations vs. time profiles. Interestingly, a recent study suggested that patients receiving higher topotecan doses may develop pharmacokinetic interactions with this combination [24]. Thus, the advantage of the metronomic schedule is that it may avoid unfavorable drug interactions that are likely dose dependent.

16.2.2 Metronomic Microtubule-Binding Agents

16.2.2.1 Vinorelbine

Microtubule-binding agents have been suggested to be the most promising cytotoxic drugs for metronomic administration because of their ability to suppress microtubule dynamics and interfere with endothelial cell functionality at very low concentrations [25–27].

Oral vinorelbine, a semisynthetic vinca alkaloid with antimicrotubule activity, has been administered metronomically in clinical studies on lung and breast cancers [10, 28–30]. The availability of this oral formulation (soft caps) is clearly advantageous for chronic, metronomic administration. The pharmacokinetics of oral vinorelbine at standard doses has been described as linear with a moderate interpatient variability, showing a bioavailability of 40 %, which is not influenced by food or age. Oral vinorelbine is rapidly absorbed (1.5–3 h) with an elimination half-life of approximately 40 h, and it shows a low level of binding to plasma proteins (13 %), whereas it is highly bound to platelets (78 %). Oral vinorelbine is metabolized mainly in the liver by the CYP3A4 isoform and eliminated mainly in an unconjugated form via the bile [31].

The pharmacokinetics of metronomic oral vinorelbine were described by Briasoulis and colleagues in 2009 [11]. In this open-label, ascending-dose (from 20 to 70 mg given thrice a week) trial, 62 patients were enrolled, but only 37 were tested for vinorelbine blood concentrations and included into the pharmacokinetic evaluation. Samples were collected after 14 days from the beginning of the treatment and up to 5 months after the beginning of metronomic regimen. Also low-dose vinorelbine showed linear pharmacokinetics with a constant concentration/dose ratio and a proportional increase of concentrations for escalating administered doses. Moreover, the blood concentration steady state for both vinorelbine and its active metabolite 4-O-deacetylvinorelbine was achieved after 2 weeks of treatment, and it was stable for months, ranging from 0.5 to 1.5 ng/ml [11]. Interestingly, the achieved steady-state concentrations were consistent with the previous in vitro findings evaluating the optimal inhibition of endothelial cell proliferation [32], supporting the hypothesis that the chosen schedule of oral vinorelbine was able to attain protracted, very low, but cytotoxic concentrations for endothelial cells [11]. In 2013, the results of a multi-institutional randomized open-label phase IB trial were published by the same group [10]. Seventy-three patients were randomly assigned to 30, 40, or 50 mg vinorelbine, taken orally three times a week, and the pharmacokinetics of the drug was performed. Trough levels of vinorelbine were measured in

blood samples from 44 patients over a time that ranged from 2 to 36 weeks. Steady-state concentrations were similar to those previously obtained [11], with no evidence of accumulation over time. Indeed, the measured mean concentration values were 1.8 ± 1.10 ng/ml (for the 30 mg dose), 2.2 ± 1.87 ng/ml (40 mg), and 2.6 ± 0.69 ng/ml for the 50 mg dose [10]. Thus, both vinorelbine and its active metabolite achieved steady-state concentrations at the low nanomolar range, which were found in vitro to preferentially inhibit the proliferation of endothelial cell and induce the expression of endogenous antiangiogenic molecules.

16.2.2.2 Paclitaxel

At standard doses, paclitaxel binds to the beta-subunit of polymerized tubulin and inhibits the dissociation rate of the tubulin subunits from the tubule. Besides these known pharmacodynamic properties, paclitaxel also exhibits antiangiogenic activity [27]. In the last decade, numerous efforts have focused on improving the pharmacokinetic behavior of paclitaxel, synthesizing a variety of nanoparticle carrier systems such as liposomes, pegylated liposomes, proteins, and polymeric nanoparticles [33].

The antitumor and antiangiogenic effects of metronomic cyclic NGR (Asn–Gly–Arg)-modified liposomes containing paclitaxel (NGR-SSL-PTX) have been recently demonstrated in a preclinical model of HT1080 (human fibrosarcoma cells) tumor-bearing SD rats in vivo [34]. Thus, Luo and colleagues performed a pharmacokinetic study of metronomic NGR-SSL-PTX in a subgroup of rats, showing that paclitaxel in NGR-SSL-PTX was more slowly eliminated from the circulation. The value of the mean residence time and the elimination half-life in the NGR-SSL-PTX treatment groups significantly increased if compared with those in the standard paclitaxel treatment group. Furthermore, the bioavailability and the AUC values were significantly increased in the NGR-SSL-PTX treatment groups, whereas the clearance of paclitaxel in the NGR-SSL-PTX treatment groups was significantly lower [34].

Recently, an oral solid dispersion formulation (ModraPac001) of paclitaxel for use in low-dose metronomic chemotherapy was clinically tested in a proof-of-concept clinical study [13]. Over a period of 2 weeks, four patients received once a week 30 mg paclitaxel p.o. and 100 mg ritonavir p.o. Paclitaxel was formulated as a solid dispersion formulation (ModraPac001, 10 mg capsule) or as a premix solution. In this study, the paclitaxel mean peak plasma concentration (C_{max}) after weekly administration of 30 mg ModraPac001 was 41.8 ng/ml; but after 24 and 48 h, the plasma concentrations were 1.67 ± 098 ng/ml and 0.80 ± 0.72 ng/ml, respectively [13]. Interestingly, these concentrations resulted well within the anti-endothelial range of paclitaxel showed by the studies of Bocci et al. [35] and Wang et al. [36] and below the myelosuppression threshold of 43 ng/ml established by Gianni and colleagues [37].

16.2.3 Metronomic UFT

UFT, a combination of tegafur, a prodrug of 5-fluorouracil (5-FU), and uracil, has demonstrated clinical activity in many tumors and, in particular, in the treatment of gastrointestinal cancers [38, 39]. It has been successfully tested using low-dose

Table 16.2 Pharmacokinetic parameters of tegafur, 5-FU, 5-FUH_2, GHB, and uracil in 27 patients administered with metronomic UFT, cyclophosphamide, and celecoxib

Parameter (u nits)	Day 1	Day 28	Day 56
Pharmacokinetic parameters for FT			
AUC (h·μg/ml)	6.286[a,b] ± 5.976	15.25 ± 9.953	12.50 ± 11.04
T_{max} (h)	1.160 ± 1.405	1.479 ± 1.536	1.188 ± 1.232
C_{max} (μg/ml)	1.976[a,b] ± 1.916	4.342 ± 2.516	3.458 ± 2.965
Pharmacokinetic parameters for 5-FU			
AUC (h·μg/ml)	1.735 ± 1.712	2.221 ± 2.444	2.192 ± 2.249
T_{max} (h)	1.240 ± 1.473	1.196 ± 1.126	1.306 ± 1.296
C_{max} (μg/ml)	0.734 ± 0.732	0.851 ± 0.817	0.918 ± 1.008
Pharmacokinetic parameters for 5-FUH2			
AUC (h·μg/ml)	2.462 ± 2.368	2.884 ± 2.041	2.998 ± 1.682
T_{max} (h)	1.180 ± 0.912	1.217 ± 0.877	1.342 ± 0.867
C_{max} (μg/ml)	1.151 ± 1.121	1.179 ± 1.506	1.625 ± 1.127
Pharmacokinetic parameters for uracil			
AUC (h·μg/ml)	5.437 ± 5.726	5.658 ± 5.192	5.192 ± 5.540
T_{max} (h)	0.920 ± 0.932	1.717 ± 1.744	1.500 ± 1.496
C_{max} (μg/ml)	2.710 ± 3.833	2.123 ± 1.835	2.182 ± 2.181
Pharmacokinetic parameters for GHB			
AUC (h·ng/ml)	500.9[a,b] ± 54.75	361.1 ± 48.05	395.1 ± 60.48
T_{max} (h)	1.880 ± 1.502	2.00 ± 1.559	1.925 ± 1.558
C_{max} (ng/ml)	161.7[a,b] ± 94.55	127.7 ± 72.25	128.2 ± 75.13

[a]day 1 vs. day 28
[b]day 1 vs. day 56
[c]day 28 vs. day 56

protocols in a randomized phase III adjuvant therapy trial of non-small cell lung cancer [40] where the drug was taken orally every day for at least 2 years.

The pharmacokinetics of metronomic UFT have been recently described by Allegrini and colleagues in a subset of metastatic, fluoropyrimidine-resistant patients with advanced refractory gastrointestinal cancers enrolled in a phase II clinical trial [4]. Furthermore, this study described a statistical relationship between pharmacokinetic parameters and the clinical efficacy of the metronomic chemotherapy. The therapeutic schedule was established using, on day one, a single administration of cyclophosphamide (CTX) 500 mg/m^2 as i.v. bolus and, from day two, administration of 50 mg cyclophosphamide p.o. once daily plus 100 mg UFT p.o. and 200 mg celecoxib (CXB) p.o. twice a day. From day two, the treatment was continued without interruption. The pharmacokinetic analyses of tegafur (FT), 5-fluorouracil (5-FU), 5-dihydro-5,6 fluorouracil (5-FUH_2), uracil, and GHB were performed in 27 patients of 38 enrolled at the days 1, 28, and 56 after the start of therapy (Fig. 16.3). A statistically significant difference in the values of area under curve (AUC) and maximum plasma concentration (C_{max}) on day 1 compared with those on day 28 and day 56 of tegafur and 5-FU was found (Table 16.2 and Fig. 16.2a, b). Moreover, after the first intake of 100 mg UFT tablet, the analysis revealed a significant difference between the pharmacokinetic parameters of patients in progressive disease (PD) and in stable disease (SD) in 5-FU AUC and C_{max} values on

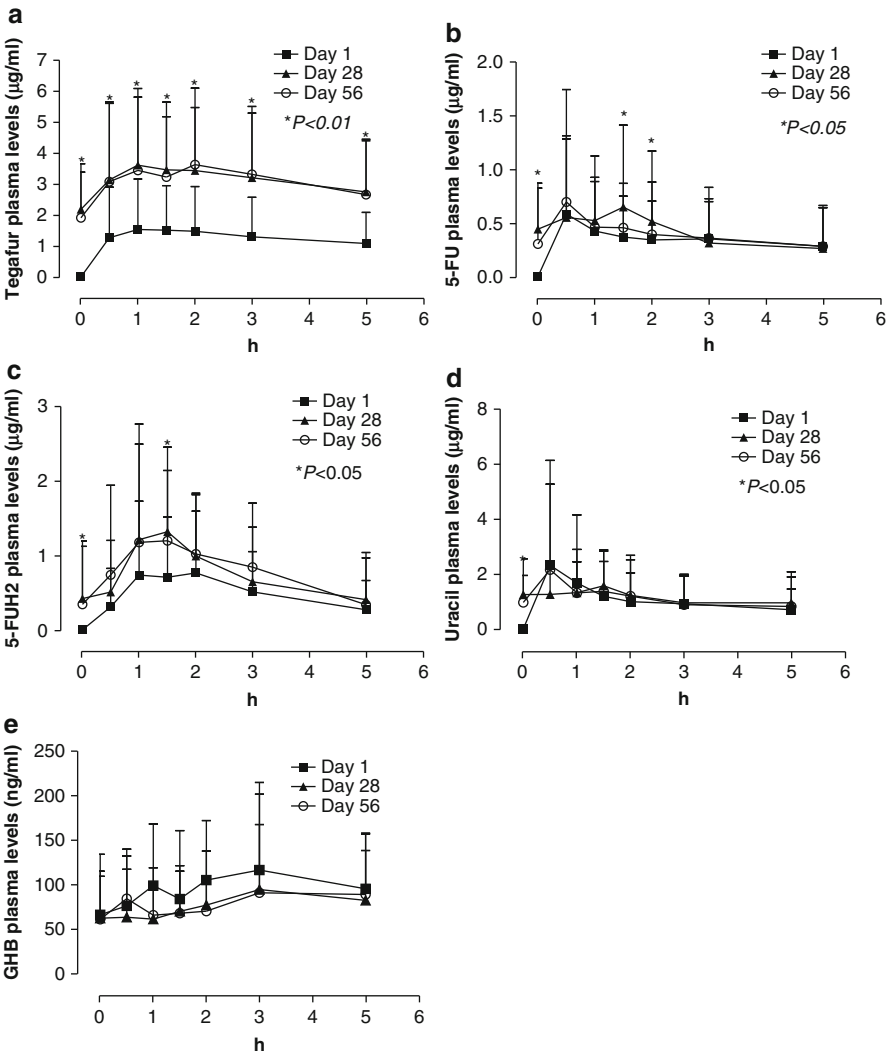

Fig. 16.2 Plasma levels of tegafur (**a**), 5-FU (**b**), 5-FUH_2 (**c**), uracil (**d**), and GHB (**e**), in 27 patients at days 1, 28, and 56, receiving the metronomic CTX, UFT, and CXB schedule. *Points* mean, *bars* standard deviation. *$P < 0.01$ and < 0.05 vs. day one values

day one (Table 16.3 and Fig. 16.3). Even more interesting, patients with the 5-FU AUC and C_{max} pharmacokinetic parameters at day one greater than the cut-off values of 1.313 h × µg/ml and 0.501 µg/ml, respectively, showed a significant prolonged progression-free survival and a significant increase in overall survival [4].

Despite the limitation of this analysis due to the small number of patients, these results identified a pharmacokinetic cut-off value in a clinically relevant population and may reveal how UFT pharmacokinetic parameters may be used from the very

Table 16.3 Pharmacokinetic parameters of tegafur, 5-FU, 5-FUH_2, GHB, and uracil in 13 patients with stable disease (SD) and 14 patients with progressive disease (PD) administered with metronomic UFT, cyclophosphamide, and celecoxib

Parameter (units)	Day 1	Day 28	Day 56
Pharmacokinetic parameters for FT (PD)			
AUC (h·μg/ml)	5.380[b] ± 6.701	17.01 ± 11.66	10.24 ± 12.72
T_{max} (h)	1.464 ± 1.770	1.833 ± 1.614	1.375 ± 1.597
C_{max} (μg/ml)	1.602[b] ± 2.122	4.900 ± 2.918	2.668 ± 3.287
Pharmacokinetic parameters for FT (SD)			
AUC (h·μg/ml)	6.295[b,c] ± 5.330	13.49 ± 8.027	14.75 ± 9.055
T_{max} (h)	0.654[c] ± 0.625	1.125 ± 1.432	1.000 ± 0.738
C_{max} (μg/ml)	2.076 ± 1.713	3.784 ± 2.009	4.249 ± 2.494
Pharmacokinetic parameters for 5-FU (PD)			
AUC (h·μg/ml)	0.997[a] ± 1.271	1.307[a] ±1.109	1.916 ± 1.702
T_{max} (h)	1.308[a] ± 1.588	1.333 ± 1.420	0.714 ± 0.636
C_{max} (μg/ml)	0.453 ± 0.573	0.542 ± 0.737	0.887 ± 1.314
Pharmacokinetic parameters for 5-FU (SD)			
AUC (h·μg/ml)	2.765 ± 1.709	3.514[d] ± 2.875	2.369 ± 2.602
T_{max} (h)	1.273 ± 1.421	1.045 ± 0.723	1.682 ± 1.488
C_{max} (μg/ml)	1.134 ± 0.749	1.188 ± 0.795	0.938 ± 0.832
Pharmacokinetic parameters for 5-FUH2(PD)			
AUC (h·μg/ml)	2.053 ± 2.361	3.170 ± 2.422	2.971 ± 1.974
T_{max} (h)	1.143[b,c] ± 1.117	1.333 ± 0.835	1.563 ± 1.116
C_{max} (μg/ml)	0.903 ± 1.027	2.111 ± 1.898	1.887 ± 1.496
Pharmacokinetic parameters for 5-FUH2 (SD)			
AUC (h·μg/ml)	2.983 ± 2.382	2.573 ± 1.582	3.018 ± 1.537
T_{max} (h)	1.227 ± 0.607	1.091 ± 0.944	1.182 ± 0.643
C_{max} (μg/ml)	1.466 ± 1.203	1.291 ± 0.802	1.435 ± 0.790
Pharmacokinetic parameters for uracil (PD)			
AUC (h·μg/ml)	4.217 ± 4.810	4.343 ± 5.037	4.855 ± 6.064
T_{max} (h)	0.893 ± 1.022	1.625 ± 1.760	1.750 ± 1.648
C_{max} (μg/ml)	1.928 ± 2.368	1.597 ± 1.618	1.479 ± 1.586
Pharmacokinetic parameters for uracil (SD)			
AUC (h·μg/ml)	6.989 ± 6.625	7.093 ± 5.201	5.417 ± 5.429
T_{max} (h)	0.954 ± 0.850	1.818 ± 1.807	1.333 ± 1.435
C_{max} (μg/ml)	3.705 ± 5.101	2.697 ± 1.957	2.651 ± 2.453
Pharmacokinetic parameters for GHB (PD)			
AUC (h·ng/ml)	491.5 ± 302.2	303.1 ± 256.5	422.5 ± 235.3
T_{max} (h)	2.143 ± 1.537	1.625 ± 1.479	2.25 ± 0.9258
C_{max} (ng/ml)	165.9 ± 114	107.7 ± 75.35	144.8 ± 61.98
Pharmacokinetic parameters for GHB (SD)			
AUC (h·ng/ml)	512.9 ± 74.34	424.4 ± 20.21	376.9 ± 86.7
T_{max} (h)	0.5667 ± 2.524	1.328 ± 3.49	0.230 ± 0.753
C_{max} (ng/ml)	111.4 ± 201.5	105.7 ± 193.2	64.03 ± 170.1

[a]PD vs. SD
[b]day 1 vs. day 28
[c]day 1 vs.day 56
[d]day 28 vs. day 56

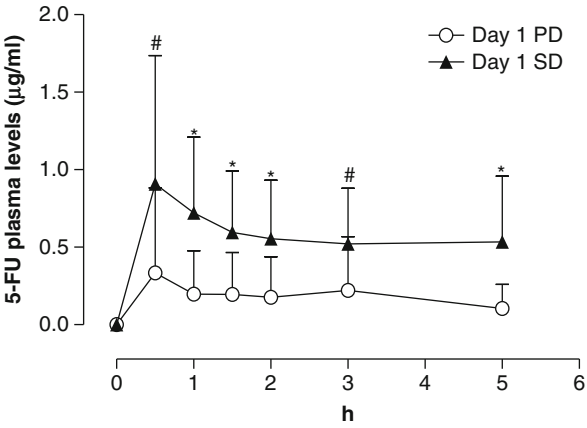

Fig. 16.3 Plasma levels of 5-FU in 13 stable disease (*SD*) patients and 14 progressive disease (*PD*) patients at day 1, 28, and 56, receiving the metronomic CTX, UFT, and CXB schedule. *Points* mean, *bars* standard deviation, $^{*}P<0.05$ PD vs. SD, $^{\#}P<0.01$ PD vs. SD

first administration of the drug to predict the efficacy and the survival of colorectal patients undertaking the metronomic schedule. Interestingly, as in the case of other chemotherapeutic drugs, the observed 5-FU concentrations are far lower than those that can be achieved with conventional 5-FU chemotherapeutic schedules.

16.2.4 Metronomic Alkylating Agents

Although alkylating drugs such as cyclophosphamide (CTX) and temozolomide (TMZ) are among the most commonly used compounds for metronomic regimens administered in the clinic to treat various tumor types such as breast, prostate, and brain cancers [41, 42], few clinical pharmacokinetic data are currently available. Reasons for this include methodological issues such as long-term sampling or low-sensitivity detection methods. They also include the neglect of the importance of the relationship between plasma concentrations of metronomic drugs (and their active metabolites) and clinical activity. This has consequently led to the absence of such data for the commonly used 50 mg/day cyclophosphamide schedule.

16.2.4.1 Cyclophosphamide

The preclinical pharmacokinetics of metronomic cyclophosphamide were investigated in xenotransplanted mice and in tumor-free animals of the same strain [43]. The concentrations of one active metabolite of cyclophosphamide (i.e., 4-OH-cyclophosphamide) were measured in the blood of three different mouse strains that were continuously given 20 mg/kg/day of cyclophosphamide through the drinking water for up to 8 weeks [44]. The authors found that the steady-state 4-OH-CTX concentrations were reached after 1 week and that the active metabolite levels measured after 8 weeks of metronomic administration were similar to those after 1 week of treatment, suggesting the absence of accumulation phenomena. The variability in AUC and C_{max} values among the mouse strains was ascribed to the interstrain heterogeneity of CTX biotransformation. Of note, the presence

of PC-3 xenografts resulted in decreased 4-OH-CTX concentrations in nude mice compared with tumor-free animals of the same strain [43].

Currently, no clinical data are available on metronomic CTX pharmacokinetics in adult patients. Adenis and colleagues recently performed a 3 + 3 dose-escalating phase I trial with a fixed dose of metronomic cyclophosphamide (50 mg two times daily) plus imatinib (400 mg per day; 300 and 400 mg two times daily), studying the imatinib pharmacokinetic parameters. The authors concluded that no dose-limiting toxicity and no drug–drug pharmacokinetic interaction were observed [45].

16.2.4.2 Temozolomide

TMZ is rapidly and well absorbed after oral administration, and it undergoes spontaneous hydrolysis at physiological pH to form its active metabolite, 3-methyl-(triazen-1-yl) imidazole-4-carboxamide (MTIC), which further degrades to 5(4)-aminoimidazole-4(5)-carboxamide and a highly reactive methyl-diazonium cation. Zhou and colleagues made a comparative pharmacokinetic study in nude rats using conventional and metronomic doses of TMZ to provide a foundation for the design of optimal metronomic TMZ treatments [46]. The pharmacokinetics of TMZ appeared linear and both dose and time independent, as there were no differences between the systemic clearance and the volume of distribution in the conventional and metronomic dose groups on the first day and the last treatment day. The ratio of the mean AUC values on day one in the conventional dose group to those in the metronomic dose group was 5.6, which was identical to the dose ratio. In addition, the $t_{1/2}$ of TMZ remained essentially the same, independently from the dose and time of sampling. Interestingly, the authors discovered that there were no sustainable changes in tumor accumulation of the drug between the conventional and metronomic dose regimens [46].

Baruchel and colleagues demonstrated in 2006 the feasibility and safety of administering metronomic TMZ in pediatric cancer patients, and they also determined a TMZ pharmacokinetic profile in these children [14]. The pharmacokinetic study was conducted in 19 patients on day one of the first cycle at various time points after the TMZ dose. The peak concentration and the area under the curve increased with increasing doses, and TMZ at metronomic doses showed a linear pharmacokinetics, although with an important interpatient variability due to a limited sample size in each cohort and various dose levels. The C_{max} ranged from a value of 2.42 ± 0.61 mg/l after the administration of 50 mg/m^2 TMZ dose to 3.51 ± 1.26 mg/l after 100 mg/m^2 TMZ dose where the AUC varied from 10.66 ± 7.70 to 13.66 ± 4.64 mg/l·h, respectively [14]. No correlation was observed between pharmacokinetic data (AUC and peak concentration) and toxicity of or response to TMZ.

16.2.5 Future Perspectives on Pharmacokinetics of Metronomic Chemotherapy

The lack of a well-known pharmacokinetic profile represents the “dark side” of metronomic chemotherapy regimens and makes it impossible to determine, among other things, (i) an optimal biological dose, (ii) the correct dose reduction vs. the

conventional schedules, and (iii) any possible pharmacokinetic interactions with other drugs, such as tyrosine kinase inhibitors, or therapeutic antibodies that target angiogenic proteins.

Moreover, if significant research effort is not devoted to this specific area of pharmacology research, it will be impossible to (i) determine the main mechanisms of action involved in the success of metronomic chemotherapy at a specific range of drug concentrations in plasma or (ii) identify valid pharmacodynamic markers of the therapy in oncology patients for such drug concentrations. Indeed, although some objective responses have been obtained in various tumors, it remains mainly unknown which plasma concentrations of the drugs were attained in the responding subjects. This makes it difficult to objectively evaluate the value of the metronomic administration of chemotherapeutic drugs. Thus, randomized clinical trials that integrate pharmacokinetic analysis are absolutely essential to better evaluate the clinical benefit of metronomic chemotherapy for the palliative treatment of cancer.

16.3 Pharmacogenetics of Metronomic Chemotherapy

Optimum drug administration is important not only for ensuring good patient outcomes in clinical practice but also in the design of clinical trials during drug development. The costs of the clinical development of a new drug or a novel therapeutic regimen are enormous, and therefore, it is critical that all drug candidates selected for human trials should be evaluated in the most efficient, cost-effective manner. With drugs used in the field of metronomic chemotherapy having unknown therapeutic indices, it becomes imperative that we understand the mechanisms behind the observed variability in drug response when treating a cancer patient.

Pharmacogenetics, an important component of individualized therapy in cancer patients, focuses on describing the extent to which an individual's genetic background is responsible for the observed differences in drug efficacy and toxicity profiles [2]. This information is then used to make predictions about the toxicity and efficacy of chemotherapeutic drugs in patients. Inherited variability of drug targets, drug-metabolizing enzymes, and drug transporters may all have a major impact on overall drug response, disposition, and associated adverse drug reactions by altering the pharmacokinetic and pharmacodynamic properties of chemotherapeutic drugs [47]. Single-nucleotide polymorphisms (SNPs) are the simplest and most commonly studied DNA polymorphism that occurs in the human genome, and they account for more than 90 % of the genetic variation observed between individuals.

Although metronomic chemotherapy has been used for over a decade in patients, it has not yet been investigated from a pharmacogenetics perspective, with the exception of two pilot studies [48, 49]. Indeed, new pharmacogenetics approaches to predict the clinical effects of metronomic chemotherapy regimens and the survival of patients are urgently needed in order to improve the personalization of this therapy for cancer patients. The role of the tumor microenvironment in the response to antitumor therapies is being increasingly emphasized [50]. Indeed, the individual genetic background of patients could have an important role in the responses to

chemotherapeutic drugs or to antiangiogenic agents, such as metronomic chemotherapy, by modulating the secretion of pro-angiogenic factors (e.g., IL-8 or VEGF) or of endogenous angiogenesis inhibitors (e.g., TSP-1). Schultheis and colleagues performed a very interesting research on 70 recurrent/metastatic ovarian cancer patients, who were treated with metronomic CTX and bevacizumab [49]. Patients harboring the IL-8 + 251 AA or AT genotypes had a significantly lower response rate than those with the TT genotype, whereas patients with the VEGF-A +936CT genotype showed a trend (although not statistically significant) for longer median progression-free survival, compared with those with the TT genotype [49]. Thus, these results may suggest that the IL-8 251A/T polymorphism could be a molecular predictor of response for the combination therapy of metronomic CTX and bevacizumab.

A recent study focused on the VEGF-A gene and its genetic variants in order to evaluate their influence on the response and survival to metronomic CTX therapy in 43 patients with metastatic prostate cancer [48]. Orlandi and colleagues tested the hypothesis that VEGF-A functional polymorphisms could modulate the response of some prostate cancers to metronomic treatment. Therefore, the study of VEGF-A SNPs should help identify those patients that are susceptible to, or resistant to, metronomic therapy. In that study, in nonresponder patients, the –634CC VEGF-A genotype frequency was 22.73 %, whereas no patient with CC genotype was observed in the responder's group ($P = 0.0485$). Moreover, the -2578CC VEGF-A genotype resulted more frequent (18.60 % vs. 2.33 %) in nonresponders ($P = 0.0212$). However, the most relevant finding of that pharmacogenetics pilot study was the identification of a VEGF-A genotype that was significantly associated with progression-free survival. Indeed, patients harboring the -634CC VEGF-A genotype had a median PFS of 2.2 months (95 % CI 0.45–3.95 months), whereas patients with genotype –634CG/GG VEGF-A had a median PFS of 6.25 months (95 % CI 3.28–8.62 months; $P = 0.0042$) [48]. Thus, a genetically determined modulation of VEGF-A in the tumor microenvironment could have a decisive role in the response of a tumor to metronomic therapy.

The validation of specific polymorphisms that will predict response to metronomic regimens is a complex process, which will need to involve both pilot and randomized clinical studies. At the present time, metronomic chemotherapy is mainly explored as a palliative treatment strategy after numerous lines of standard chemotherapy in phase II clinical trials and few phase III studies have been planned [41]. In that respect, the collaborative efforts of investigators who actively worked in this field will be particularly important in providing a wider series of patients to validate promising but preliminary results. For example, future phase III metronomic clinical trials should include analysis of a full coverage of genes and genetic variants of the IL-8 and VEGF-A pathways, based on the available preliminary data [48, 49]. Moreover, future studies should also include the analysis of SNPs of genes involved in the metabolism of chemotherapeutic drugs, such as CYP2B6, 3A4, and 2C9 involved the biotransformation of CTX into a 4-hydroxycyclophosphamide, the so-called activation step [51]. Indeed, SNPs that could enhance or decrease the enzymatic activity of the above-described CYPs may vary the tumor response to

metronomic chemotherapy and could therefore have an impact on the survival of patients treated with such regimens.

Pharmacogenetics analyses should be conducted as an integral part of large randomized phase II/III trials of metronomic chemotherapy or as independent studies that focus on the validation of specific genetic determinants. However, the introduction of pharmacogenetics of metronomic chemotherapy into clinical practice will be very difficult, even if candidate genes (e.g., IL-8 or VEGF-A) will be characterized. Indeed, studies aimed at documenting clinically the predictive efficacy of such SNPs, and a comparison of pharmacogenetically guided vs. standard patient care will also be necessary.

Conclusions

The field of metronomic chemotherapy adds another level of complexity to issues such as the characterization of clinically relevant pharmacokinetic parameters or the germ-line and somatic mutations that can affect drug efficacy. However, as we begin to unravel and accurately identify (i) the mechanism of action of metronomic anticancer drugs at the real plasma concentrations obtained from low-dose regimens and (ii) the polymorphisms in candidate genes likely to influence drug efficacy, we may start to consider the personalization of metronomic chemotherapy regimens for cancer patients.

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